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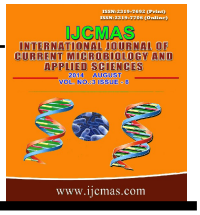

Original Research Article

Physiological, Spectroscopic and Biochemical Analyses of Effect of Solar Visible and UV Radiation on the Cyanobacterium, *Lyngbya majuscula*

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Keywords

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A B S T R A C T

The effects of some components of solar radiation on the photosynthetic oxygen production, pigmentation and phycobiliprotein composition were investigated in the cyanobacterium, *Lyngbya majuscula.* The organism was exposed to unfiltered solar radiation (UV-B, UV-A and PAR), and solar radiation filtered through optical filters, WG320 (UV-A and PAR), GG400 (PAR only), and UG5 (only UV-B and UV-A). Photosynthetic oxygen production was impaired in the organism with this occurring within 45 minutes. The effect due to unfiltered solar radiation and combined UV-A and PAR were severe. Absorption spectrum of the crude extract of *Lyngbya* indicated the presence of chlorophyll *a* as the photosynthetic pigments, carotenoids, phycoerythrin and phycocyanin as the accessory pigments, which were bleached under the various treatments. Generally, the phycobilins were affected most, but carotenoids had the shortest half-life, followed by the phycobilins and then chlorophyll *a*. Sucrose gradient ultracentrifugation also revealed allophycocyanin as an additional accessory pigment *Lyngbya*. Fluorescence measurements showed peaks which significantly decreased in amplitude and also underwent a shift towards shorter wavelengths, with prolonged exposure time indicating a reduction in their energy transfer ability. This study reveals that the various components of solar radiation adversely affected photosynthetic oxygen production, pigmentation and protein composition in *Lyngbya* and that the effect due to the combined visible and uv radiations had a drastic effect than only visible or only UV.

Introduction

The ultimate source of energy for the biosphere is the sun. Even though the sun emits the whole of the electromagnetic spectrum, due to reflection, molecular and particulate scattering and absorption of radiation in the atmosphere, only 23% of UV-B, 72% of UV-A and 91% of the visible extraterrestrial radiation reach the surface of the earth (Häder and Tevini, 1987). The solar spectrum at the ground surface can be partitioned according to the biological effectiveness of the major wavebands. These are ultraviolet (UV) radiation (100-400 nm) and the visible radiation waveband (400-700 nm), which is also referred to as photosynthetically active radiation (PAR).

PAR is important in photosynthesis and plant photomorphogenesis, although it also has significant thermal and photodestructive effects (Ross, 1975). The next is the infrared (IR) region which extends from 700 nm to approximately 4000 nm and is primarily significant in regard to thermal effects on organisms.

The ultraviolet radiation is further divided into UV-A (320-400 nm), UV-B (280-320 nm) and UV-C (100-280 nm). The UV-A portion of the spectrum is relatively harmless, but UV-B has many damaging effects while UV-C is lethal to many forms of life (Acevedo and Nolan, 1993). The invisible UV radiation is effectively absorbed by nucleic acids (affecting the genome), proteins and pigments. Ozone forms a thin layer in the stratosphere and acts as a filter for UV radiation from the sun, removing most of UV-C and part of UV-B.

The natural formation and breakdown of ozone is in equilibrium and thus result in an effective shield against injurious UV radiation at the ground. However, there are reports of increasing UV-B reaching the earth's surface as a result of anthropomorphic damage to the stratospheric ozone layer (Crutzen, 1992; Weiler and Penhale, 1994). There are concerns of the risks posed by this to ecosystems. Marine algae inhabiting the intertidal zone are exposed in a short time span to sharp fluctuations of their physical environment. Enhanced solar ultraviolet radiation (UVR) due to stratospheric ozone depletion is a major stress factor for many phototrophic organisms in aquatic and terrestrial ecosystems (Franklin and Forster, 1997). Solar UV radiation is among the major factors that determine the physiological performance of these organisms at intertidal rocky shores (Huovinen and Gómez, 2011).

Experiments have shown that the photosynthetic pigments are bleached within minutes or hours when exposed to unfiltered solar radiation (Häder *et al*., 1989; 1991; Donkor and Häder, 1991) and by UV-B radiation (Nultsch and Agel, 1986; Häder et al., 1988; and Häder and Worrest, 1991). Even though UV-A irradiation is not photosynthetically active, it can be a potent force regulating biological productivity in aquatic systems, capable of impairing nutrient assimilation, photosynthesis, and photo repair of molecular damage in algae (Zhou *et. al*. 2009; Srinivas and Ochs, 2013).

Most reported studies of aquatic organisms and ecosystems concern the Antarctica and surrounding marine waters and other temperate regions. Also most of the organisms used are isolates cultured in the laboratory. It is therefore imperative that studies are carried out on the effect of solar radiation on naturally-occurring cyanobacteria found in tropical aquatic ecosystems, since tropical ecosystems are exposed to greater fluxes of UV radiation even in the absence of ozone depletion. The aim of this study was to examine the effect of solar visible and ultraviolet radiation on the photosynthesis, pigment and proteins in this cyanobacterium found in the tropical coastal marine waters in Ghana.

Materials and Methods

Organism used

Lyngbya majuscula is a marine, filamentous cyanobacterium used for this study.

Collection of organism

The organism was collected from the intertidal zone of the shore on the coast of the Gulf of Guinea (Atlantic Ocean) at

Biriwa (5 09'N,1 10'E), about 10 kilometres from Cape Coast in the Central Region of Ghana.

It was collected during low tides either between 6.00 and 7.00 a.m. on the day of measurements or in the evening before the day of measurement. The harvested organism was temporarily kept in beakers containing sea water (which was also fetched whenever samples were collected), in a laboratory at the Botany Department of the University of Cape Coast. The samples were aerated using an aspirator, and kept under white light from fluorescent lamps.

Identification of organism

The samples were thoroughly washed in sea water and all debris, particles of sand and any foreign body removed with the help of a hand lens. Temporal slides of the organisms were prepared and mounted on a microscope equipped with a JVC Colour video camera, by which the image of the organism was transmitted unto a JVC video screen for close observation of features and examination of characteristics of interest.

The organisms were identified comparing the observed characteristics with available literature on the classification of blue-greens (Desikachary, 1959; Drouet, 1968; Pentecost, 1984; Lawson and John, 1987).

Exposure to solar radiation (treatments)

The organisms were exposed in open glass petri dishes of diameter 5 cm. These were kept in an ice bath to prevent excessive heating around the organism, maintaining a temperature of about $26^{O}C$, whilst the ambient temperature was $38^{O}C$. The blue-green algae were exposed to either unfiltered sunlight or covered with one of three cut-off filters:

(i) WG 320 cut-off filter, which transmits radiation above 320 nm, thus permitting photosynthetically active radiation (PAR) and UV-A to pass through;
(ii) GG400, which transmits radiation above 400 nm, thus allowing only PAR to pass through and;
(iii) UG5, a UV passing filter which allows radiation in the range of 250 and 400 nm (i.e. UV-A and UV-B) to pass through.

All the filters were 3 mm thick. The organisms were exposed to solar radiation between 10.00 a.m. and 2.00 p.m. Samples were removed at regular intervals during irradiation for the various analyses. The unirradiated sample (those that were not exposed to solar radiation) served as control.

Measurement of solar irradiance

Solar irradiance was recorded during the experimental period at the department of Botany, University of Cape Coast, in order to determine the solar irradiance reaching the organisms. The fluence rates for the components of solar radiation (UV-A, UV-B and visible) were determined with a Radiometer. The Gossen Mavolux digital luxmeter was used to measure the solar radiation intensity in Watts per square metre between 10.00 a.m. and 2.00 p.m. The measurements were taken at thirty-minute intervals.

Photosynthetic oxygen exchange

The photosynthetic oxygen production was determined using a Clark electrode (Dubinsky *et al*., 1987). 100 ml of sea water was poured into a 150 ml conical flask and 0.5 g of the organism transferred into it and then agitated with a magnetic stirrer. This was enough to give measurable changes in oxygen concentration over short periods of time. A 250-Watt slide projector which was

equipped with a halogen lamp was used as a light source for the photosynthetic measurements. A Clark oxygen electrode connected to a microprocessor OXI-meter was inserted (free of air bubbles) into the flask. This was adjusted to prevent self-shading by the filament from occurring in the electrode assembly. The whole assembly was covered with a black cloth to prevent light diffusion and any external light source influence. The photosynthetic activity of the cyanobacterial samples was measured before and after regular intervals of solar irradiation. Dark respiration was recorded after the light from the slide projector had been switched off.

Extraction of photosynthetic pigments

0.5 g of the organism was removed at regular intervals during solar irradiation, and grounded using a mortar and a pestle. 3 ml of a 0.75 M phosphate buffer (pH 7.5, made up of $K_2 HPO_4$ and $KH_2 PO_4$) was added. To the homogenate, 60 µl of l mM PMSF solution (in Iso-propanol) was also added to prevent protein breakdown through protease action. The suspension was poured into a centrifuge tube. A further 3 ml of the phosphate buffer was used to rinse the mortar and added to the suspension. 30 µl of Triton X-100, a detergent was then added to the suspension and mixed thoroughly. The Triton X-100 solubilized the membrane to allow the phycobilins to come out easily. The resulting suspension was incubated for 30 minutes at room temperature.

The suspension was then centrifuged at 6000 rpm for 15 minutes, using the H-1500 FR Centrifuge to remove large fragments and cell debris. The supernatant (the crude extracts) was carefully poured into a test tube and subjected to absorption, fluorescence, sucrose gradient centrifugation and gel electrophoresis analyses.

Sucrose gradient centrifugation

The supernatant (crude extract) obtained after the extraction of the photosynthetic pigments was subjected to a step sucrose gradient centrifugation to separate the phycobiliproteins (phycobilins). The 10%, 25% and 40% step density gradient in phosphate buffer, found to be the most suitable, was used for this study. With the help of a micropipette, 1 ml of 40% sucrose in the phosphate buffer was transferred into centrifuge tube, followed by 1 ml of 25% and then 10% sucrose in the phosphate buffer. 1 ml of the crude extracts of the sample was carefully introduced on top of the sucrose gradient. The tubes were put in a swinging bucket RPS 56T-146 Rotor and then centrifuged for 2 hours at 40000 rpm at $5^{O}C$ in an Ultracentrifuge. The resulting fractions were carefully retrieved from the tubes into a 1 ml syringe and subjected to absorption and electrophoretic analyses.

Absorption spectrum

The crude extracts of the organism before and after regular intervals of exposure to solar radiation, were each transferred into 3 ml plastic cuvettes with optical path lengths of 10 mm (2 mm thickness). The absorbance was measured using a spectrophotometer. The absorption spectrum for the organism was determined at an interval of 5 nm from 350 nm to 700 nm to determine the peaks of the major photosynthetic pigments. For the isolated protein fractions, this started from 400 to 700 nm. Subsequent absorption values for exposed samples were determined at the peak wavelengths which indicated the maximum absorption of the pigments. In order to evaluate the effect of bleaching the values for the control was subtracted from the subsequent values.

Fluorescence

Fluorescence excitation and emission spectra of the extracted pigments of the organism were measured in a Spectrofluorophotometer. These measurements were taken before and after exposure to solar radiation at various time intervals.

Experimental design and statistical analysis

The Randomized Block Design with replicates was used for this study. 0.5 gram of the organism was weighed and transferred into the small petri dish (5 mm in diameter). Each petri dish contained four pieces of 0.5 g of the organism. The petri dishes were randomly covered with three (3) filters, leaving one with no filter. The components of solar radiation served as treatments, with duration of exposure to solar radiation being the blocks. The unirradiated sample served as the control. Analysis of variance was carried out on the data obtained and Duncan's multiple range test used to separate the means (Duncan, 1955; Ott, 1993).

Results and Discussion

Description and identification of the cyanobacterium

The organism used for this study was filamentous. The thallus was dull blue-green to brown but violet when dried. The trichomes occurred singly in a thin firm colourless sheath. The filaments were attached at the base to rock surfaces that were covered with sand deposits and were loosely together. The trichome was 34 μm broad and about 4 cm long. The trichomes were cylindrical and exhibited gliding movement over one another. The trichomes were neither constricted at the cross walls, nor truncated at the ends. Rather, the apex appeared rounded. The organism lacked a nuclear membrane, and also the membranes bearing photosynthetic pigments were dispersed in the cytoplasm, not arranged in chloroplast. The organism possessed neither mitochondria nor heterocysts. From these characteristics and available literature (Desikachary, 1959; Drouet, 1968; Pentecost, 1984 and Lawson and John, 1987), the organism was identified as *Lyngbya majuscula* (hereafter referred to as *Lyngbya*).

Photosynthetic oxygen production

The fluence rates' range of the components of solar radiation during this experimental period were: 805 - 1010 Wm^{-2} (Total sunlight), 658 - 875 Wm^{-2} (Visible radiation), 20.9 - 26.5 Wm^{-2} (UV-A) and 3.4 - 4.8 Wm^{-2} (UV-B) (Fig. 1). Generally, the photosynthetic rate increased after 15 minutes of exposure to the various treatments, followed by a decrease with increasing exposure time (Fig. 2). Under unfiltered radiation (PAR, UV-A and UV-B), the decrease in the photosynthetic rate after the initial increase was drastic in *Lyngbya*, from 0.5 mg l^{-1} min^{-1} to -0.2 mg l^{-1} min^{-1} after 30 minutes of exposure (Fig. 2a). When exposed to PAR and UV-A, the photosynthetic rate started decreasing after 15 minutes of exposure, reaching -0.2 mg l^{-1} min^{-1} after 45 minutes and maintained this rate even after 60 minutes of exposure (Fig. 2b). Similar results were obtained when exposed to PAR only, with a final rate of -0.1 mg l^{-1} min^{-1}. The rate of dark respiration was not affected (Fig. 2b) and was similar to those exposed to PAR and UV-A. Under UG 5 (UV-A and UV-B), there was a remarkable increase in the photosynthetic rate from 0.2 to 0.6 mg l^{-1} min^{-1} after 15 minutes of exposure. Thereafter there was a

gradual reduction reaching -0.1 mg l^{-1} min^{-1} after 60 minutes of exposure time (Fig. 2d).

Measurement of absorption spectra

The absorption spectrum of the crude extract of *Lyngbya* showed peaks which indicated the major photosynthetic pigments as chlorophyll *a* at 440 nm and 670 nm, carotenoids with a peak at 495 nm and that of phycoerythrin and phycocyanin being 565 nm and 620 nm respectively (Fig. 3). The effect of solar radiation on the pigments was investigated by taking the absorbance maxima corresponding to the photosynthetic pigments. Generally, all these pigments showed a decrease in absorbance with prolonged exposure times to the various treatments. The initial absorption values of the peaks of the unirradiated control were subtracted from all subsequent values after increasing exposure to solar radiation. This showed the differences in absorption at the peaks, giving the kinetics of the bleaching (Fig. 4).

It was observed that under the various treatments, carotenoids had the shortest half-life, followed by phycocyanin, phycoerythrin and then chlorophyll *a*. The thalli that received unfiltered radiation (PAR, UV-A and UV-B) was affected most, followed by those which received combined PAR and UV-A, and then visible radiation only (PAR) and combined UV-A and UV-B (Fig. 4).

In vitro fluorescence

The fluorescence emission spectra of *Lyngbya* when excited at 575nm (Fig.5) and 615 nm (Fig. 6) both showed an emission peak at 640 nm. Generally the amplitude of the peak decreased with increasing solar irradiation, accompanied by shift to shorter wavelengths. After one hour of exposure, the fluorescence peaks of the pigments had declined significantly for those exposed to PAR, UV-A and UV-B and those that received combined PAR and UV-A ($p< 0.05$). Thereafter there was a gradual decrease with those exposed to PAR, UV-A and UV-B expressing the most affected effect (Figs. 5 and 6).

The effect caused by PAR only and combined UV-A and UV-B followed similar trends. However, the decreases in the peaks were quite gradual. There was a drastic decline after 2 hours of exposure. The intensity of the peak after 4 hours was not significantly different between the treatments. This is so for the fluorescence emission spectra of *Lyngbya* excited at 575 nm and 615 nm (Figs. 5 & 6).

Sucrose gradient centrifugation

The crude extract of the unirradiated sample of *Lyngbya* after sucrose gradient ultracentrifugation yielded 3 distinct coloured fractions. These fractions were (from the top) Fraction 1 (greenish-yellow): Chlorophyll a (440 nm and 670 nm), Fraction 2 (light-red): Phycoerythrin (565 nm) and Phycocyanin (615 nm), Fraction 3 (blue/purple): Phycoerythrin (565 nm) and Phycocyanin (615 nm) and Allophycocyanin (650 nm).

The absorption spectra of the fractions obtained after sucrose gradient centrifugation indicated that unfiltered solar radiation had a drastic effect on the pigments. The effects of the various treatments on fraction 2 of *Lyngbya* are shown in figure 7. Unfiltered radiation drastically affected the phycoerythrin. Two peaks were identified in samples exposed to UV-A and PAR for 1 and 2 hours (Fig. 7b), PAR only, for 3 and 4 hours (Fig. 7c), and only UV-A and UV-B, for 3 and 4 hours (Fig. 7d).

Investigations carried out in this study showed that exposure of *Lyngbya* to the various components of solar radiation, adversely affected photosynthesis oxygen exchange, pigmentation and proteins, an indication that the cyanobacterium was sensitive to solar radiation. The photosynthetic capacity of the cells, measured using a constant white light after increasing exposure to the various components of solar radiation, was affected. All wavelength bands of solar radiation (UV-B, UV-A and visible) impaired the photosynthetic capacity in *Lyngbya* indicating that inhibition of photosynthesis occurred.

Exposure of *Lyngbya* to unfiltered solar radiation resulted in a drastic decline in the rate of oxygen production after an initial increase. Photosynthesis stopped after about 22 minutes as the thalli showed only net respiration. Thereafter, the inhibition of photosynthesis was less drastic when exposed to UV-A and PAR. The net respiration was evident after 37 minutes. The effect was similar to samples exposed to only PAR. Under both UV-A and UV-B, the net respiration was evident only after 50 min. of exposure. This result is in accordance with studies conducted by Leverenz *et al*. (1990) and Helbling *et al*. (1992), who demonstrated that exposure of phytoplankton to solar radiation of high fluence rates causes photoinhibition that is characterized by a reduction in the quantum yield and the photosynthetic oxygen evolution. Donkor and Hader (1991; 1996) have noted that the inhibition of photosynthesis is even more rapid than the effect on other physiological processes in cyanobacteria such as motility and orientation. According to Häder (1996), many species when exposed to strong solar irradiation show photoinhibition or even photodamage, which is caused, in part, by visible radiation and to a larger extent by UV.

Photoinhibition, which is a widespread phenomenon among photosynthetic organisms (Powles, 1984), can be determined by oxygen exchange measurements (Field *et al*., 1989). It is known that the mechanisms of photoinhibition are employed by organisms to regulate their photosynthetic activity to operate under limited light supply and to protect themselves against excessive radiation (Hanelt *et al*.,1993). It is known that both visible and UV radiation inhibits photosynthesis. According to Walsby (1968) the effects of visible radiation on photosynthesis depends on the intensity of the radiation. The photosynthetic system is limited at low irradiance, saturated at high irradiance and inhibited at very high irradiance (Hanelt et. al., 1993). After 1 hour of exposure of *Lyngbya*, to the various treatments, it was observed that UV-B had the most adverse effect on the organism. The result confirms that of Larkum and Wood (1993) who observed that using artificial irradiation, the drop in photosynthetic oxygen production caused by UV-B radiation in various species of phytoplankton is stronger than inhibition due to PAR.

According to Barber and Anderson (1992), exposure to an excess of white light causes the formation of singlet oxygen, mediated by the triplet state of the P_{680}, with the consequent inhibition of electron transfer through PS II, the proteolytic degradation of the D1 – polypeptide and eventually, the disassembly of the PS II complex. It is also known that exposure to UV, has a deleterious effect on the photosynthetic apparatus, leading to a reduction in the supply of ATP and $NADPH_2$ (Kulandaivelu and Noorudeen, 1983; Kumar *et al*., 1996).

Cellular components of the thylakoid membrane are degraded by exposure of cyanobacteria to UV and that UV wavelengths cause an inactivation of the PS II reaction (Noorudeen and Kulandaivelu, 1982), while PS I appears to be more resistant (Iwanzik *et al.*,1983; Agel *et al.*,1987). It has been established that exposure to UV-B radiation causes damage to the photosynthetic apparatus in many species of algae (Franklin et al., 2003; Kitzing *et al*., 2014), mainly damage of the thylakoid photochemistry and related processes, which can finally lead to a decrease in oxygen evolution, electron transport, Rubisco activity and finally, CO_2 fixation rates. (Gomez *et al*., 2007).

The difference in the absorption peaks gives the kinetics of the bleaching. It shows the rate at which pigments corresponding to those peaks were getting bleached. The difference in the absorption at the peaks of the photosynthetic pigments of *Lyngbya* showed that the pigments were significantly ($p < 0.01$) bleached by solar UV and visible radiation. This agrees with data obtained by other researchers who worked with cyanobacteria. Nultsch and Agel (1986), working with *Anabaena variabilis*, observed that photobleaching in the cyanobacteria was induced by visible light. A similar observation has been reported by Häder et al.(1988). Donkor and Häder (1996) in their work with *Anabaena*, *Oscillatoria teunis*, *Nodularia* and *Phormidium* found that photobleaching of photosynthetic pigments can also be induced by exclusive UV radiation. The percentages of bleaching give an indication of how much bleaching has occurred at any instant of time. In *Lyngbya*, the carotenoids seemed to be bleached faster, followed by the phycobilins and then chlorophyll. Carotenoids have been implicated in UV and bright light photoprotection of surface blooms of freshwater cyanobacteria (Paerl et al., 1983) in addition to their function of energy transduction to the chlorophyll reaction centres. Therefore if they are bleached at this rate then it is likely to have a damaging effect on the cyanobacterium. The low values of absorption by carotenoids may be attributed to the reduced rate of its biosynthesis. As reported by Häder and Worrest (1991), it has been shown that UV radiation can photo-oxidize and thereby bleach all types of photosynthetic pigments and may also cause a depression of chlorophyll *a* and carotenoids via reduced rates of biosynthesis. A decrease in the phycobiliprotein contents and disassembly of phycobilisomal complex following UV irradiation have been reported by Sinha et al (1995a, 1995b) in a number of rice field cyanobacteria, indicating impaired energy transfer from the accessory pigments to the photosynthetic reaction centers. Cruces and others (2013) reported that, short-term exposures between 2 and 6 hours to enhanced UV radiation and temperature were effective to activate the photochemical and biochemical defences against oxidative stress in *Lessonia nigrescens* and *Durvillaea antarctica.*

Most of the excitation energy absorbed by the phycobilins is passed to chlorophyll *a*, however, *in vivo* fluorescence spectra indicates that the phycobilins show a considerable auto-fluorescence even in the unirradiated control samples. This indicates that the excitation energy cannot be utilized by the photosynthetic apparatus effectively. Generally, in *Lyngbya*, the spectroscopic analyses showed a significant decline in the fluorescence peaks accompanied by a shift to shorter wavelengths. This indicates that the absorbing pigments were progressively being destroyed by exposure to solar radiation, impairing energy transfer from phycobilisomes to the photosynthetic reaction centres.

Fig.1 Fluence rates of components of solar radiation between 10:00 hours and 14:00 hours of the day

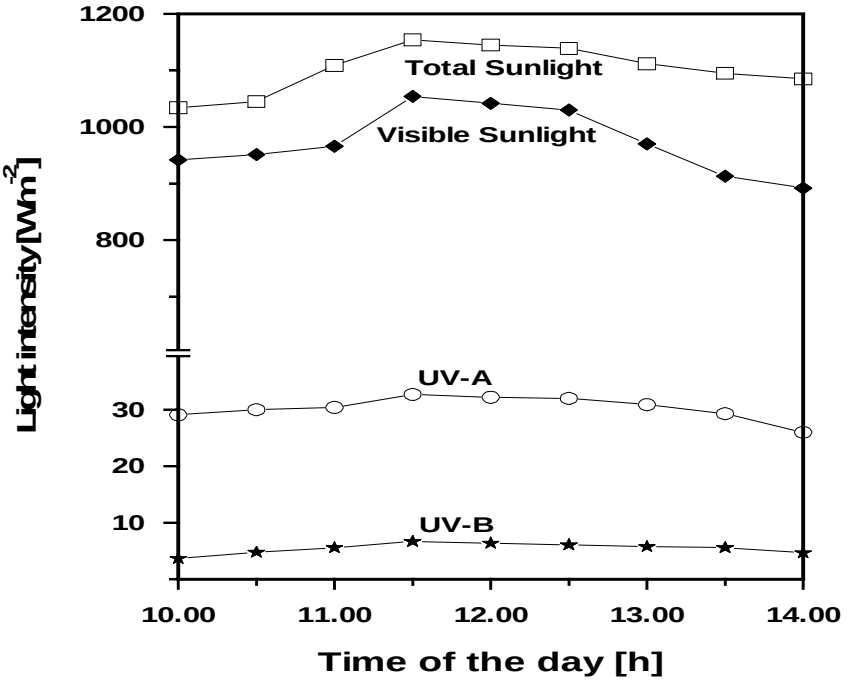


Fig.2 Photosynthetic oxygen production (solid lines) and dark respiration (broken lines) of *Lyngbya* after increasing times of exposure to (a) PAR + UV-A + UV-B (b) PAR + UV-A (c) PAR and (d) UV-A + UV-B

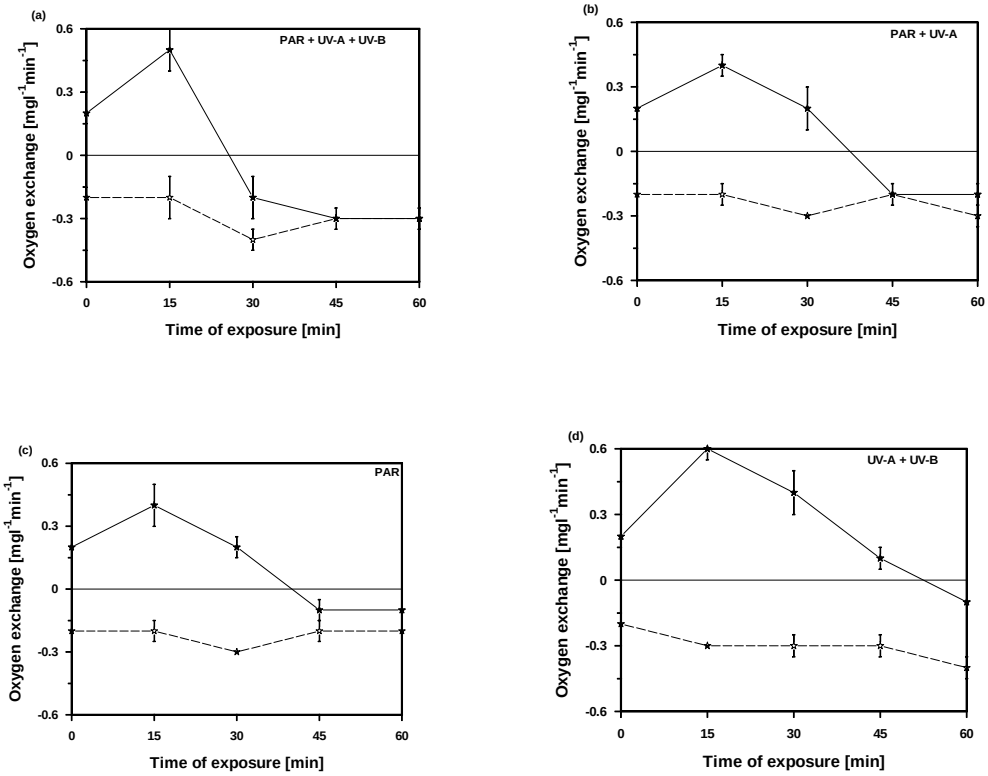


Fig.3 Absorption spectrum of *Lyngbya sp*. showing absorption maxima corresponding to the photosynthetic and accessory pigments

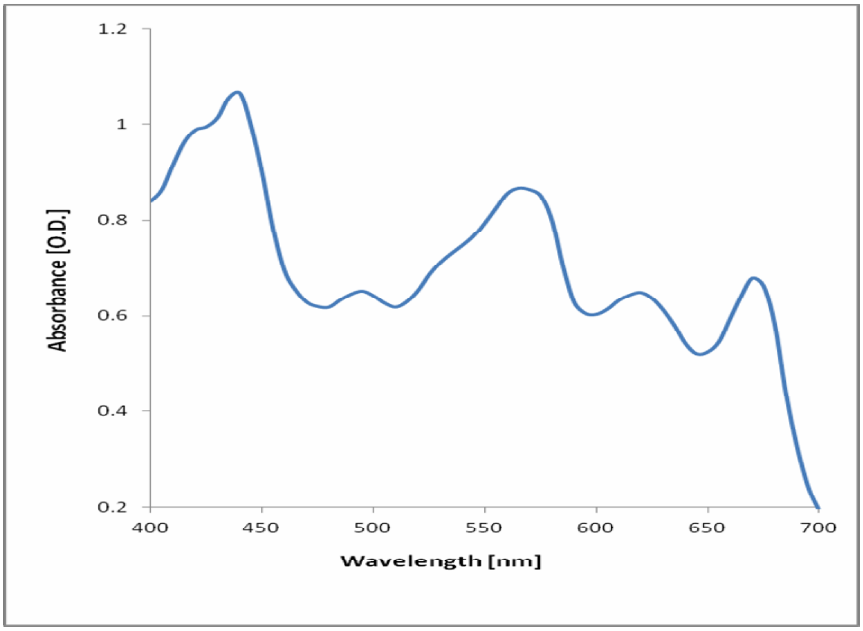


Fig.4 Kinetics of bleaching of photosynthetic pigments of *Lyngbya* after increasing times of exposure to (a) PAR + UV-A + UV-B (b) PAR + UV-A (c) PAR and (d) UV-A + UV-B

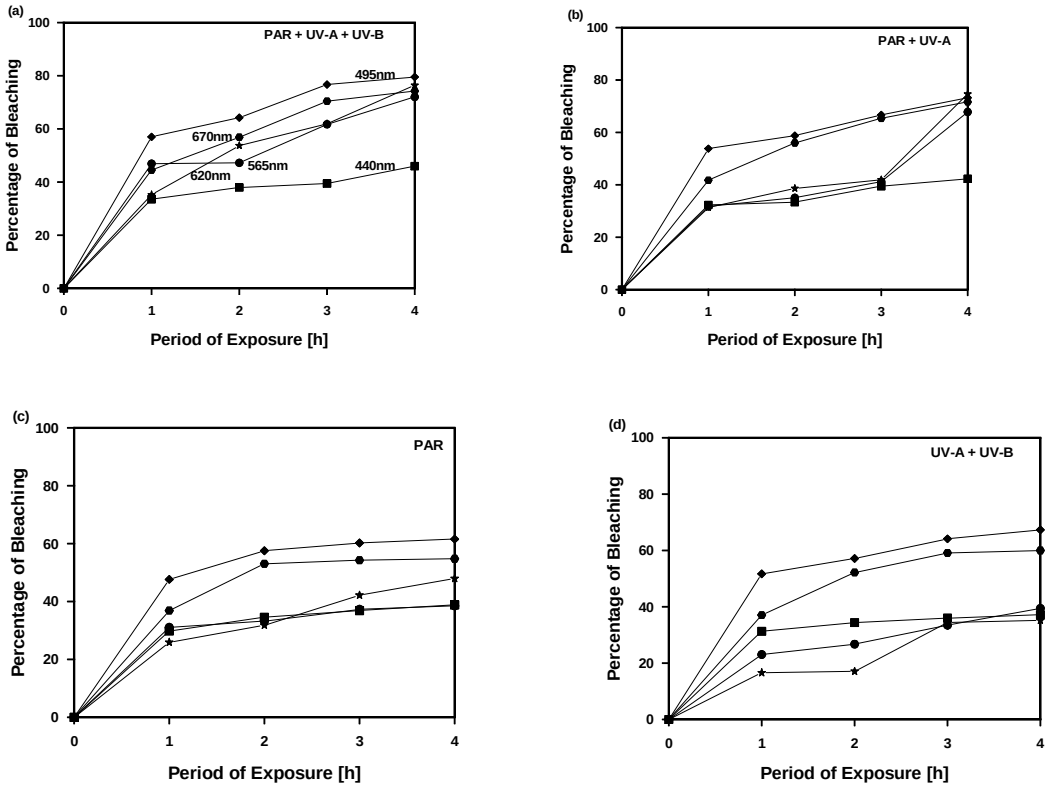


Fig.5 Fluorescence emission spectra of *Lyngbya* monitored at an excitation wavelength of 575 nm after increasing times of exposure to (a) PAR + UV-A + UV-B (b) PAR + UV-A (c) PAR and (d) UV-A + UV-B

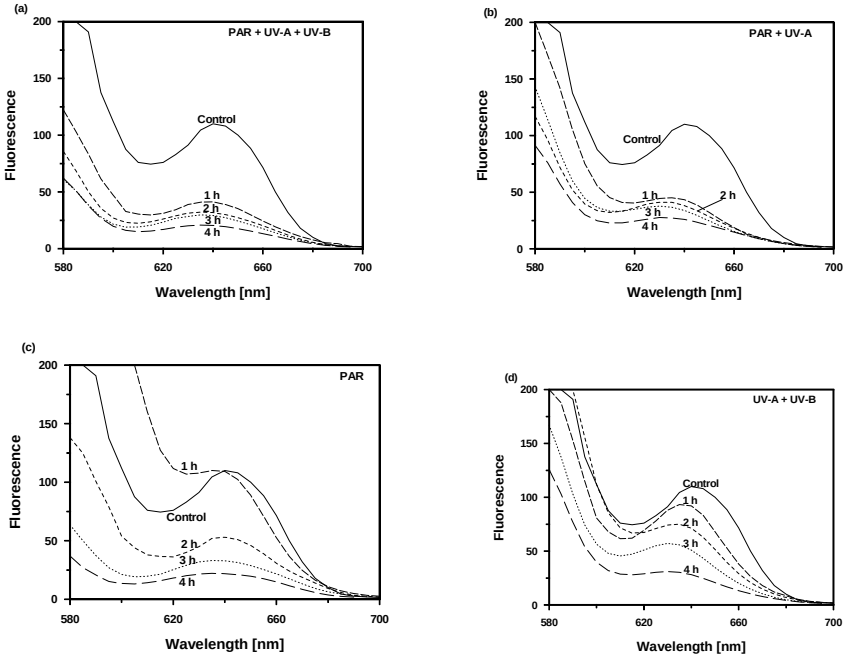


Fig.6 Fluorescence emission spectra of *Lyngbya* monitored at an excitation wavelength of 615 nm after increasing times of exposure to (a) PAR + UV-A + UV-B (b) PAR + UV-A (c) PAR and (d) UV-A + UV-B

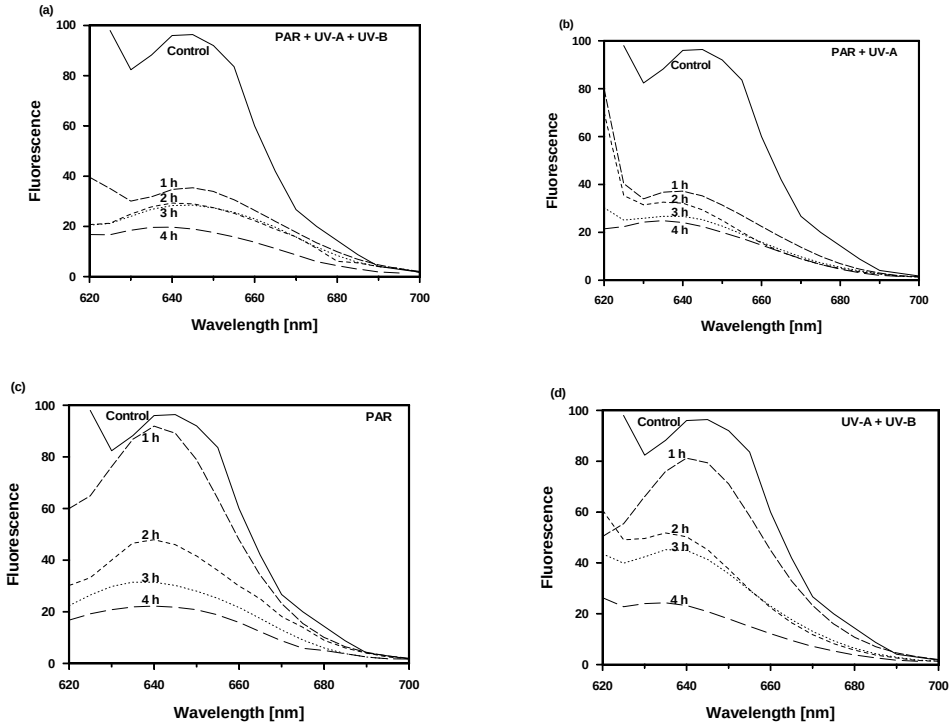
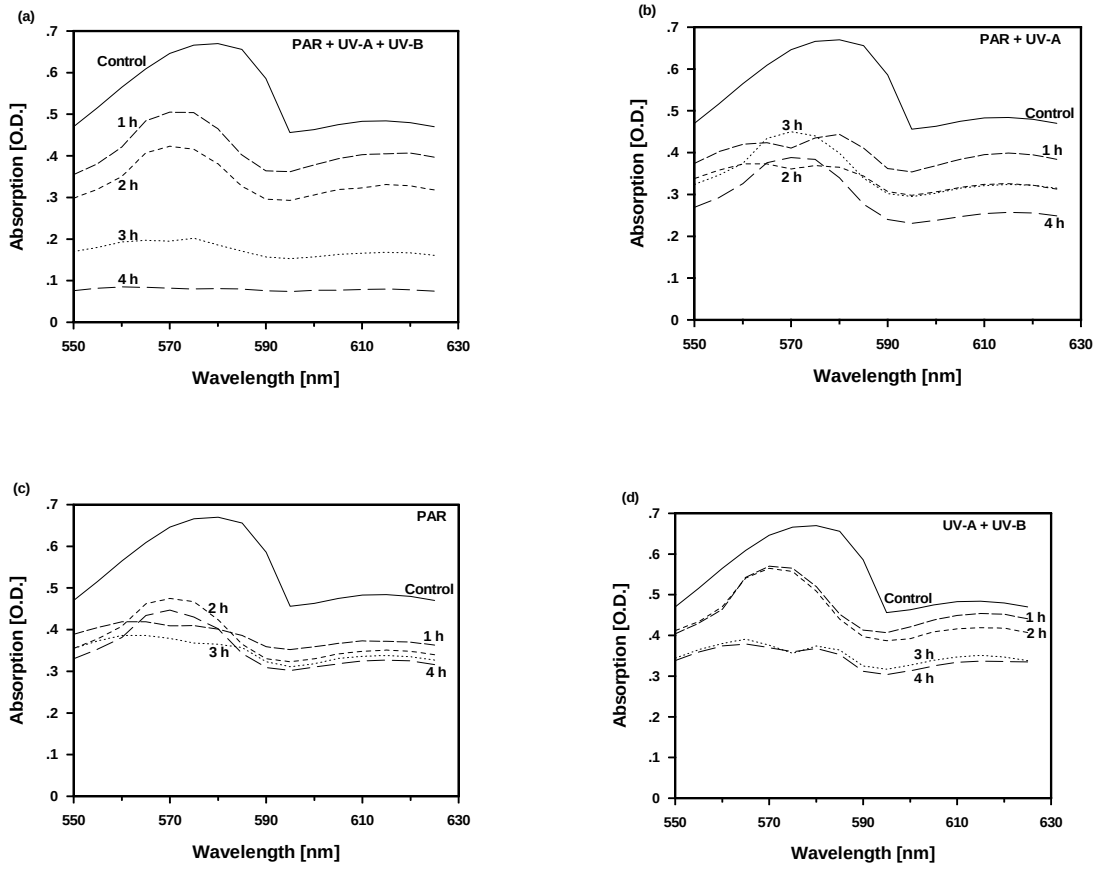


Fig.7 Absorption spectra of fraction 2 of *Lyngbya* after sucrose gradient centrifugation after increasing times of exposure to (a) PAR + UV-A + UV-B (b) PAR + UV-A (c) PAR and (d) UV-A + UV-B



The shift of the emission maximum to shorter wavelengths observed in *Lyngbya* could be due to the occurrence of multiple charge isomers of phycocyanins as observed in *Chroomonas* species (Mörschel and Wehrmeyer, 1975). Hill and Rowan (1989), have also demonstrated multiple forms of phycoerythrin in *Cryptomonas* sp, absorbing at 545nm (type I), 555 nm (type II) and 565-568 nm (type III) which could be separated by isoelectric focussing (IEF). The shift in emission peaks under irradiation thus indicates a photoconversion into other phycoerythrin and phycocyanin charged isomers. That is, the sequential appearance of these forms during exposure to solar radiation indicates that these charged isomers may be degradation products of an initial form.

In vivo, the phycobiliproteins are arranged in high-molecular mass aggregates and structurally composed in phycobilisomes (Gantt and Conti, 1966), which in electron microscopic studies have been found to be

attached to photosystem II particles and arranged in rows on the surface of the thylakoids in cyanobacteria and red algae (Mörschel and Rhiel, 1987). The subunits are connected by linker proteins (Glazer, 1982). It has been suggested that the first step in phycobilisome disintegration is a breakdown of the supramolecular structure and that UV irradiation first causes the high molecular mass aggregates to breakdown into hexamers $(\alpha\beta)_6$, which further disintegrate into trimers $(\alpha\beta)_3$ and eventually to monomers $(\alpha\beta)$ (Mörschel et al, 1980; Glazer, 1982). The absorption spectra of the fractions obtained from sucrose gradient ultracentrifugation indicated that in addition to phycoerythrin and phycocyanin, allophycocyanin was also present as an accessory pigment in *Lyngbya*.

It was observed that in samples of *Lyngbya* exposed under unfiltered radiation, there was an increase in the intensity of the colouration of fraction 3, after 1 hour of exposure. This was followed by a gradual decrease of this fraction, with increasing exposure time, with a corresponding increase in fraction 2. It may thus be suggested that the heavier proteins were being degraded to lightier ones. Sinha et al. (1995) found a close correlation in the appearance (intensity) of the protein bands in the sucrose gradient and the measured absorption spectra confirming the degradation. Fractions with high intensity had higher absorption spectra while those low in intensity had low absorption spectra. The absorption spectra of the fraction 2 of *Lyngbya* after sucrose gradient centrifugation (Fig. 7) indicated that, unfiltered radiation drastically affected the phycoerythrin. Two peaks were identified in samples exposed to UV-A and PAR for 1 and 2 hours, PAR only for 3 and 4 hours, and UV-A and UV-B combined for 3 and 4 hours (Fig. 7).

References

Acevedo, J. and Nolan, C. (eds.) 1993. Environmental UV Radiation [Causes - Effects - Consequences] European Commission, Bruxelles, Belgium. pp 9-33.

Agel, G., Nultsch, W. and Rhiel, E. 1987. Photoinhibition and its wavelength dependence in the cyanobacterium *Anabaena variabilis*. *Arch. Microbiol*. 147: 370-374.

Barber, J. and Anderson, B. 1992. Too much of a good thing: Light can be bad for photosynthesis. *TIBS*, 17: 61-66.

Cruces, E., Huovinen, P. and Gómez, I. 2013. Interactive effects of UV radiation and enhanced temperature on photosynthesis, phlorotannin induction and antioxidant activities of two sub-Antarctic brown algae. Marine Biology. 160 (1): 1 – 13.

Crutzen, P. J. 1992. Ultraviolet on the increase. *Nature* 365 :104-105.

Desikachary, T. V. 1959. *Cyanophyta*. Indian Council of Agricultural Research, New Delhi, India. pp. 4.

Donkor, V. and Häder, D.-P. 1991. Effects of solar and ultraviolet radiation on motility, photo movement and pigmentation in filamentous, gliding cyanobacteria. *FEMS Microbiol. Ecol*. 86: 159-168.

Donkor, V. and Häder, D.-P. 1996. Effects of ultraviolet irradiation on photosynthetic pigments in some filamentous cyanobacteria. *Aquatic Microbial Ecology*. 11: 143-149.

Drouet, F. 1968. *Revision of the classification of the Oscillatoriaceae*. Academy of National Sciences of Philadelphia, Pennsylvania. Monograph 15, pp. 1-370.

Dubinsky, Z., Falkowski, P. G., Post, F. and vas Hes, U. M. 1987. A system for measuring phytoplankton photosynthesis in a defined light field with an oxygen electrode. *J. Plankton Res*. 9: 607-612.

Duncan, D. B. 1955. Multiple Range and Multiple F Tests. *Biometrics* 11: 1-42.

Field, C. B., Ball, J. T. and Berry, J. A. 1989. Photosynthesis: principles and field techniques. In: *Plant Physiological Ecology* (Pearcy, R. W., Ehleringer, J., Mooney, H. A. and Rundel, P. W., eds.) Chapman and Hall, London. pp. 209-253.

Franklin, L.A. and Forster, R.M. 1997. The changing irradiance environment: consequences for marine macrophyte physiology. Eur. J. Phycol. 32: 207-232.

Franklin, L.A., Osmond, C.B., Larkum, A.W.D. 2003. Photoinhibition, UV-B and algal photosynthesis. In: Larkum AW, Douglas SE, Raven JA (eds) Photosynthesis in algae. Kluwer, The Netherlands, pp 351–384

Gantt, E. and Conti, S. F. 1966. Phycobiliprotein localization in algae. *Brookhaven Symp. Biol.* 19: 393-405.

Glazer, A. N. 1982. Phycobilisomes: Structure and dynamics. *Ann. Rev. Microbiol.* 36: 173-198.

Gómez, I., Orostegui, M., and Huovinen P. 2007. Morpho-functional patterns of photosynthesis in the South Pacific kelp *Lessonia nigrescens*: effects of UV radiation on ^{14}C fixation and primary photochemical reactions.

Häder, D.-P. 1996. Light penetration and effects on aquatic ecosystems. In: *Light as an Energy Source and Information Carrier in Plant Physiology*. (Jennings, R. C., Zucchelli, G., Ghetti, F. and Colombetti, G. eds.) Plenum Press, New York. pp. 231-242.

Häder, D.-P. and Tevini, M. 1987. *General Photobiology*. Pergamon Press, Britain. pp. 313.

Häder, D.-P., Rhiel, E. and Wehrmeyer, W. 1988. Ecological consequences of photomovement and photobleaching in the marine flagellate *Cryptomonas maculata*. *FEMS Microbiol. Ecol*. 53: 9-18.

Häder, D.-P. and Worrest, R. C. 1991. Effects of enhanced solar UV radiation on aquatic ecosystems. *Photochem. Photobiol*. 53: 717-725

Häder, D.-P., Worrest, R. C. and Kumar, H. D. 1989. Aquatic ecosystems. In: *UNEP Environmental Effects Panel Report*. pp. 39-48.

Häder, D.-P., Worrest, R. C. and Kumar, H. D. 1991. Aquatic ecosystems. In: *UNEP Environmental Effects Panel Report.* pp. 33-44.

Hanelt, D., Hupperts, K. and Nultsch, W. 1993. Daily course of photosynthesis in marine macrophytes investigated in the laboratory and in the field. *Mar. Ecol. Prog. Ser*. 97: 31-41.

Helbling, E. W., Villafañe, V. E., Ferrario, M. and Holm-Hanson, O. 1992. Impact of natural ultraviolet radiation on rates of photosynthesis and on specific marine phytoplankton species. *Mar. Ecol. Prog. Ser*. 80: 89-100.

Hill, D. R. A. and Rowan. K. S. 1989. The biliproteins of the Cryptophyceae. *Phycologia* 28: 455-463.

Huovinen, P. and Gómez, I. 2011. Spectral attenuation of solar radiation in Patagonian fjords and coastal waters and implications for algal

photobiology. Cont Shelf Res 31:254–259.

Iwanzik, W., Tevini, M. Dohnt, G. Voss, M., Weiss, W., Graber, P. and Reger, G. 1983. Action of UV- B radiation on photosynthetic primary reactions in spinach chloroplastes. *Physiol. Plant* 58:401- 407.

Jagger, J. 1967. *Introduction to Research inUltraviolet Photobiology*. Prentice-Hall Inc. New Jersey. pp. 164.

Kitzing, C., Pröschold, T. and Karsten, U. 2014. UV-Induced Effects on Growth, Photosynthetic Performance and Sunscreen Contents in Different Populations of the Green Alga *Klebsormidium fluitans* (Streptophyta) from Alpine Soil Crusts. Microbial Ecology 67: 327-340.

Kumar, A., Sinha, R. P. and Häder, D.-P. 1996. Effect of UV-B on enzymes of nitrogen metabolism in the cyanobacterium *Nostoc calcicola*. *J. Plant Physiol.* 148: 86-91.

Kulandaivelu, G. and Noorudeen, A. M. 1983. Comparative study of the action of ultraviolet-C and ultraviolet-B radiation on photosynthetic electron transport. *Physiol. Plant* 58: 389-394.

Larkum, A. W. D. and Wood, W. F. 1993. The effect of UV-B radiation on photosynthesis and respiration of phytoplankton, benthic macroalgae and seagrass. *Photosynth. Res*. 36: 17- 23.

Lawson, G. W. and John, D. M. 1987. *The Marine Algae and Coastal Environment of Tropical West Africa* (2nd. ed.) J. Cramer in der Gebriider Borntraegar Verlagsbuchhandbung, Berlin. pp. 335-355.

Leverenz, J. W., Falk, S., Pilström, C.-M. and Samuelson, G. 1990. The effect of photoinhibition on the photosynthetic light-response curve of green plant cells (*Chlamydomonas reinhardtii*). *Planta* 182: 161-168.

Mörschel, E. and Rhiel, E. 1987. Phycobilisomes and thylakoids: the light-harvesting system of cyanobacteria and red algae. In: *Electron microscopy of proteins, Vol. 6. Membranous structures*. (Harris, J. K. and Horne, R. W. eds.) Academic Press, London. pp. 209-254.

Mörschel, E. and Wehrmeyer, W. 1975. Cryptomonad biliprotein: phycocyanin-645 from a *Chroomonas* species. *Arch. Microbiol*. 105: 153-158.

Mörschel, E. Koller, K.-P. and Wehrmeyer, W. 1980. Biliprotein assembly in the disc- shaped phycobilisomes of *Rhodella violacea* electron microscopical and biochemical analyses of C-phycocyanin and allophycocyanin aggregates. *Arch. Microbiol*. 125: 43-51.

Noorudeen, A. M. and Kalandaiveln, G. 1982. On the possible site of inhibition of photosynthetic electron transport by ultraviolet-B (UV-B) radiation. *Physiol. Plant* 55: 161-166.

Nultsch, S. and Agel, G. 1986. Fluence rate wavelength dependence of photobleaching in the cyanobacterium *Anabaena varibilis*. *Arch. Microbiol*. 144: 268-271.

Ott, R. L. 1993. *An Introduction to Statistical Methods and Data Analysis.* (4th edn.) Wadsworth Publishing Company, california, USA. pp. 767-897.

Paerl, H. W., Tucker, J. and Bland, P. T. 1983. Carotenoid enhancement and its role in maintaining blue-green algal (*Microcystis aruginosa*)

surface blooms. *Limnol. Oceanogr*. 28: 847-857.

Pentecost, A. 1984. Cyanobacteria (Blue-green Algae) In: *Introduction to Freshwater Algae*. Richmond Publishing Co. Ltd., England. pp. 18-43.

Powles, S. B. 1984. Photoinhibition of photosynthesis induced by visible light. *Annu. Rev. Plant Physiol.* 35: 15-44.

Ross, J. 1975. Radiative transfer in plant communities, In: *Vegetation and the Atmosphere,* Vol. 1 (Monteith, J. L., ed.), Academic Press. London. pp. 13-15.

Sinha, R. P., Lebert, M., Kumar, A., Kumar, H. D. and Häder, D.-P. 1995. Spectroscopic and Biochemical Analyses of UV Effects on Phycobiliproteins of *Anabaena* sp. and *Nostoc carmium*. *Bot. Acta* 108: 87-92.

Srinivas, R. and Ochs, C.2013. Effect of UV-A Irradiance on Lipid Accumulation in *Nannochloropsis oculata*. Photochemistry and Photobiology. 88: 684 – 689.

Walsby, A. E. .1968. Mucilage secretion and the movements of blue-green algae. *Protoplasma* 65: 223- 238.

Weiler, C. S. and Penhale, P. A. 1994. Ultraviolet radiation in Antarctica: measurements and biological effects. *Antarct. Res. Ser*. 62, Am. Geophys. Union. Washington DC.

Zhou, W., T. Xuexi, X. Hui, W. You *and* W. Renjum 2009. Response of marine microalgae, heterotrophic bacteria and their relationship to enhanced UV-B radiation. *J. Ocean Univ. China* 8(1): 35–38.